Measurement of Antibody-Reactive Toxin Antigen During Experimental Staphylococcal B Enterotoxemia

SIGURD J. NORMANN

Department of Pathology, University of Florida, Gainesville, Florida 32610

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Staphylococcal enterotoxin B (SEB) injected intravenously is rapidly cleared from the circulation and deposited in tissues. Type-specific antiserum administered after toxin has left the circulation can influence the course of enterotoxemia, and this observation suggests that toxin antigens may either be returned to the circulation or reside on cell surfaces readily available to antibody. If SEB toxin or its fragments regain access to the extracellular space, they might react with circulating antibody and be detected and quantitated by the reduction in antibody titer. Accordingly, 1 h after toxin or saline injection, animals were given type-specific enterotoxin B antiserum, and the difference in titers between the animals was used to compute the amount of enterotoxin immediately available to antibody. Further, by measuring differences in titers over a 48-h period, an estimate was made of the amount of SEB antigen that gained access to antibody. The data indicate that rats, which are relatively resistant to the lethal effects of enterotoxin, clear toxin from the circulation promptly and that very little toxin reenters the circulation. Monkeys, who are highly susceptible to SEB, also clear toxin promptly. However, in contrast to rats, monkeys have greater quantities of SEB immediately available to antibody and in addition return significant quantities of toxin antigens to the extracellular space.

Staphylococcal enterotoxin B (SEB) is a single-chain polypeptide (28,500 daltons) which can produce a number of signs and symptoms similar to endotoxemia on systemic administration (4, 16). While monkeys and rabbits exhibit lethargy, fever, normovolemic shock, and death in response to the toxin, the same toxin when given to rats and mice appears to have little clinical effect. However, both species of animals rapidly clear intravenously administered toxin such that 1 h after challenge there is 3% or less of the injected dose present in the circulation (1, 9). The toxin localizes in kidney tubular cells in amounts equivalent to 42 to 75% of the injected dose, with 5 to 25% in liver and 0.7 to 5% in lung (6, 9). Successful prevention of the pathological effects of enterotoxemia can be achieved by the prior administration of specific equine anti-SEB antiserum (11); it is also true that the course of enterotoxemia can be significantly altered by administration of the antiserum after exposure to the toxin. Considering this latter observation together with the very rapid clearance of the toxin from the circulation, the possibility must be entertained that enterotoxin

or its antigenic fragments are returned to the circulation from cellular reservoirs or that biologically active toxin resides on cell surfaces readily available to antibody.

By analogy to the production of hemagglutination inhibition, it appeared that one might be able to measure the amount of enterotoxin antigen readily available to circulating antibody by injecting toxin or saline followed by antiserum and measuring the initial difference in titers between the two animals. Further, the method might be able to quantitate SEB that reenters the circulation because such toxin should produce a progressive fall in titer of circulating anti-SEB antibody. SEB has been measured by a variety of techniques including hemagglutination inhibition (7), single diffusion (17), and radioimmunoassay (1), but when these methods are applied to measuring circulating SEB they depend upon measuring actual SEB blood levels and such SEB may be in transit between entry and exit from the circulation. By capturing such antigen with antibody and measuring the effect produced on the antibody titer, it might be possible to

determine the total amount of antigen that has access to circulating antibody during the course of enterotoxemia.

In the studies which follow, specific anti-SEB antiserum was injected 1 h after toxin or saline injection, and the change in serum titer in the animals was compared. The studies suggest that the monkey, which is very susceptible to SEB, has more toxin available to antibody throughout the course of enterotoxemia than does the rat, which is a relatively resistant animal.

MATERIALS AND METHODS

Highly purified SEB was obtained from the Army Medical Research Institute of Infectious Disease, Frederick, Md; and was identical to that described by Schantz et al. (13). It is a single-chain polypeptide devoid of lipid and carbohydrate whose amino acid sequence is known (4).

Specific equine anti-SEB antiserum was prepared as described by Rapoport et al. (11) by subcutaneous hyperimmunization over a 4- to 5-month period with increasing doses of SEB without adjuvant. The specific antiserum had a hemagglutination titer of 1:640,000 and formed only a single precipitin line with SEB on immunoelectrophoresis or Ouchterlony gel diffusion. The reacting antibody was identified as equine immunoglobulin (Ig)G (T) globulin (8).

Male Sprague-Dawley rats (150 to 250 g) and rhesus monkeys (2 to 4 kg) of either sex were used. None of the animals had a known history of exposure to SEB. Hemagglutination tests were performed on randomized rats as these animals rarely have hemagglutinating antibody against SEB, whereas all monkeys were screened for the absence of enterotoxin antibody prior to experimentation. The monkeys were placed in restraining chairs 2 days prior to the start of the studies. Food and water were available ad libitum except for overnight fasting on the day preceding the experiments. An indwelling cannula (PE 90) was placed in the saphenous vein under local anesthesia and extended into the posterior vena cava to facilitate intravenous injections and blood samples. Rats were anesthetized with ether and injections were made through the penile and femoral veins. Blood samples were obtained from the femoral veins bilaterally using heparin washed syringes.

Hemagglutinating anti-SEB antibody was determined by modification of the microtiter method of Stavitsky (15) using SEB coupled to washed sheep red cells with bis-diazotized benzidine (5). Titrations were incubated at 22 C for 2.5 h and read for pattern of agglutination. The highest dilution of serum that gave a positive pattern was taken as the titer. Titrations of known positive and negative sera were included as controls.

RESULTS

Following injection of anti-SEB antibody (100 μ liters/100 g rat body weight), reciprocal hemagglutination titers were determined on

multiple blood samples obtained over a 24-h period (Fig. 1). The average initial titer ± 1 standard deviation obtained in 5 rats 1 h after antiserum administration was $\log_2 = 12.2 \pm 1.3$. In the absence of enterotoxin or in the presence of saline injections of up to 2 ml, the titer tended to decrease over the ensuing 24-h period but the fall in titer was not statistically significant. The final titer was $\log_2 = 10.4 \pm 1.1$. Therefore, nearly all of the equine antibody remained within the vascular compartment of normal animals during this period. In contrast, enterotoxin, either as a single injection or as eight divided doses totaling 25 μ g/100 g, produced a marked fall in titer. In two rats following a single SEB injection, the average titer fell precipitously from $\log_2 = 12$ to $\log_2 = 4$. The titer remained relatively stable up to 24 h thereafter. When the same amount of enterotoxin was administered in eight divided doses over a 14-h period, the titer fell progressively with each SEB injection (Fig. 1). Nevertheless, at 24 h the titer was approximately the same as that obtained after a single injection. These experiments were performed in antibody excess. The method appears capable of distinguishing between toxin antigen immediately available to antibody (i.e., single injection) from antigen that reenters the circulation from a tissue reservoir (multiple injection).

FIG. 1. Comparison between serum hemagglutination titers in rats injected with saline (control) to rats injected with SEB either as single or multiple injections totaling 25 μ g of SEB/100 g. Anti-SEB antiserum was administered as 100 μ liters/100 g 1 h prior to the SEB injections. Control data is average of five rats; experimental data is average of two rats.

The capacity of hemagglutination inhibition to detect low concentrations of SEB varied with the amount of anti-SEB in the circulation. For this experiment, different amounts of antiserum were injected into separate rats followed by a series of SEB injections of 2.5, 5.0, or 10.0 $\mu\text{g}/100\text{ g}$ every 2 h. In Fig. 2, the observed serum titer after each SEB injection is plotted as a function of the cumulative enterotoxin dose for antiserum concentrations of 25, 50, 100, and 200 μliters of anti-SEB/100 g. As the concentration of antiserum increased, increased amounts of SEB were necessary not only to abolish the serum titer but also to produce a significant fall in titer. For example, 25 μliters of anti-SEB/100 g produced an initial titer of $\log_2 = 9.7$ and 10 μg of SEB/100 g abolished the titer. The same amount of SEB injected into an animal given 100 μliters of anti-SEB/100 g with initial titer of $\log_2 = 12.0$ produced a final titer of $\log_2 = 9.0$. Accordingly, the amount of antiserum administered influenced the sensitivity of the assay, yet for any given antiserum concentration, a standardized curve relating the hemagglutination titer to enterotoxin concentration could be used to estimate the amount of enterotoxin available to antibody. The necessity for a standardized curve arose from the nonlinear relationship between enterotoxin concentration and titer.

Figure 3 presents two typical experiments in which rats were given either 100 μliters or 25 μliters of anti-SEB antibody/100 g 1 h after an

FIG. 2. Relationship in rats between serum hemagglutination titer and cumulative enterotoxin injected showing the decrease in titer produced by increasing toxin concentration for four different concentrations of antiserum. The antiserum was administered as a single intravenous injection, and at 2-h intervals thereafter a blood sample was obtained followed by an injection of enterotoxin (2.5, 5.0, or 10.0 $\mu\text{g}/100\text{ g}$). Each point on the graph represents the $\log_2$ reciprocal titer observed 2 h after each enterotoxin injection plotted against the total amount of enterotoxin administered.

FIG. 3. Typical response of serum hemagglutination titers between control rats injected with saline (1 ml) and experimental rats injected with staphylococcal enterotoxin B (1,000 μg of SEB/100 g). The toxin or saline was administered 1 h prior to the antisera.

injection of 1,000 μg of SEB/100 g. When the larger amount of antiserum was administered, only a slight difference was observed between the control animal injected with saline and the experimental animal injected with enterotoxin. However, when the amount of antiserum was reduced to 25 $\mu\text{liters}/100\text{ g}$, a pronounced difference was observed. The toxin-injected rat showed a progressive fall in titer, which by 16 h was reduced to zero. During the same period, the control animal showed no significant change in titer so that at 16 h it had a titer of $\log_2 = 9$. By reference to the calibration curve (Fig. 2), 7.5 μg of SEB/100 g would abolish the titer arising from 25 μliters of anti-SEB/100 g, but 13 $\mu\text{g}/100\text{ g}$ were necessary to produce a difference of 3.6 log units at the higher concentration of 100 μliters of anti-SEB/100 g. Therefore, approximately 13 μg of enterotoxin out of every 1,000 μg injected appeared to have access to the antiserum when the latter was administered 1 h after the toxin and events were measured during the ensuing 24-h period.

Table 1 summarizes the hemagglutination inhibition data obtained in rats for a challenge dose of 1,000 μg of SEB/100 g. During the 24-h period after toxin challenge, only about 13 μg of SEB/100 g or 1.3% of the challenge dose was actually available to antibody. Since approximately 5.0 μg /100 g (0.4% challenge dose) was present at 2 h after toxin injection, reentry of enterotoxin antigen into the circulation did not exceed 8.0 μg /100 g, which is less than 0.8% of the challenge dose.

Rats are relatively resistant to the lethal effects of enterotoxin, whereas rhesus monkeys are highly susceptible. However, before measuring available enterotoxin by antibody reaction in monkeys, it was first necessary to confirm the calibration steps already performed in rats (Fig. 2). This data for rhesus monkeys is summarized in Fig. 4. First, a larger quantity of antiserum had to be administered to monkeys to produce an antibody titer equivalent to that of rats because of their greater blood volume. Second, the change in anti-SEB titer in monkeys due to SEB injection varied with the concentration of antibody in the circulation. This finding was similar to that previously noted for rats. Because of differences in blood volume, more SEB had to be given to monkeys to produce a measurable change in antibody titer.

The sequence of injections was now reversed, and enterotoxin was given intravenously 1 h prior to the anti-SEB. Figure 5 summarizes

data obtained from monkeys injected with 1,500 μg of SEB/kg for four different concentrations of anti-SEB. The injection of 100 μliters of anti-SEB/kg, which normally would produce a titer of $\log_2 = 7$, produced no measurable antibody in the sera of toxin injected animals. When the concentration of anti-SEB was raised to 250, 500, or 1,000 $\mu\text{liters/kg}$, measurable antibody was detected. When animals injected with toxin were compared to those receiving saline, noticeable changes in titer 2 h after challenge were recorded for antiserum given in amounts of 250 and 500 $\mu\text{liters/kg}$ but not for 1,000 $\mu\text{liters/kg}$. By reference to Fig. 4, the change in titer was equivalent to an average concentration of 35.8 μg of SEB/kg. Indeed, this amount of toxin should abolish the titer arising from 100 μliters of anti-SEB/kg but have marginal effect on 1,000 $\mu\text{liters/kg}$. By 8 h and 24 h, the titer arising from 250 μliters of anti-SEB/kg and 500 $\mu\text{liters/kg}$, respectively, were abolished. The titer arising from the largest amount of antiserum administered (1,000 $\mu\text{liters/kg}$) did change during the 24 h but was still measurable 36 h later.

Available antibody-reactive SEB antigen estimated over a 48-h period of enterotoxemia showed important differences between rats and monkeys as shown in Fig. 6. Whereas very little SEB antigen reappeared in the extracellular space of rats, a significant quantity did return in rhesus monkeys. For example, a rat given

TABLE 1. *Regain of SEB by the extracellular space of rats^a*

Challenge injection	Anti-SEB ^b	Log ₂ reciprocal titers		
		2 ^c	12	24
Saline	100	12.2 $\pm$ 1.33 ^d	11.2 $\pm$ 0.28 ^d	10.4 $\pm$ 1.14
SEB	100	10.6 $\pm$ 0.83	10.2 $\pm$ 1.41	6.8 $\pm$ 1.25 } 13.0 ^e
Saline	50	10.8 $\pm$ 1.81 ^d	9.8 $\pm$ 1.78	9.0 $\pm$ 1.82
SEB	50	10.0 $\pm$ 1.22	5.4 $\pm$ 1.98 } 8.0 ^e	1.0 } >7.5 ^e
Saline	25	9.7 $\pm$ 1.22	9.2 $\pm$ 1.64	7.6 $\pm$ 1.14
SEB	25	6.0 $\pm$ 0.71 } 5.0 ^e	2.4 $\pm$ 1.96 } 6.5 ^e	1.0 } >7.5 ^e
Estimated available SEB per 100 gm		5.0 μg	7.2 μg	13.0 μg
SEB-I ¹²⁵ reported in circulation (Reference 9)		<12 μg		

^a Data based on 1,000 μg of SEB/100 g and reported as average of five rats $\pm$ standard deviation.

^b Administered as μliters /100 g 1 h after challenge injection.

^c Hours after SEB.

^d No significant difference ($P < .05$).

^e Difference statistically significant with an estimate of micrograms of SEB to produce difference. The estimate was determined from the standard curve (Fig. 2). Since the titer did decrease with time in saline-injected control animals, the amount of SEB corresponding to this decrease was substrated from the amount of toxin corresponding to the titer in the enterotoxin-challenged animal.

FIG. 4. Relationship in monkeys between serum hemagglutination titer and cumulative enterotoxin injected showing the decrease in titer produced by increasing toxin concentration of four different concentrations of antiserum. The antiserum was administered as a single intravenous injection, and at 2-h intervals thereafter a blood sample was obtained followed by an injection of enterotoxin (25 or 50 $\mu\text{g}/\text{kg}$). Each point on the graph represents the $\log_2$ reciprocal titer observed 2 h after each enterotoxin injection plotted against the total amount of enterotoxin administered.

FIG. 5. Typical response of serum hemagglutination titers in monkeys injected with staphylococcal enterotoxin B (1,500 μg of SEB/kg) 1 h prior to the injection of anti-SEB antiserum. The zero time titer is that titer which should have been observed if no SEB had been in the circulation. Figures in parentheses under each antiserum concentration indicate the number of monkeys used.

1,000 μg of SEB/100 g had approximately 5 $\mu\text{g}/100$ g available to antibody at 2 h and accumulated an estimated 13 μg by 24 h and 10 μg by 48 h. Thus, the cumulative regain of toxin antigen to the extracellular space was approximately 6 $\mu\text{g}/100$ g or less than 0.6% of the injected dose. In contrast, when monkeys were injected with 1,500 $\mu\text{g}/\text{kg}$, toxin accessible to antibody at 2 h measured 36 $\mu\text{g}/\text{kg}$ (2.4% injected dose), and in the ensuing 48-h period

totaled 125 $\mu\text{g}/\text{kg}$. Thus, 89 $\mu\text{g}/\text{kg}$ reentered the extracellular space (6.9% injected dose). When the challenge dose was reduced to 500 $\mu\text{g}/\text{kg}$, monkeys had approximately 15 μg of SEB/kg available to antibody at 2 h and accumulated an estimated 50 μg of SEB/kg by 24 h and 55 μg by 48 h. Thus, toxin regain was estimated at 40 $\mu\text{g}/\text{kg}$ or 8.0% of the injected dose. Furthermore, accumulation of antibody-reactive SEB in rats appeared almost linear between 2 and 48 h, reflecting the small change that occurred; in monkeys, a rapid accumulation occurred early within the first 24 h and less was accumulated in the hours thereafter.

Table 2 summarizes survival data for monkeys challenged with SEB. In the absence of anti-SEB, the mean time to death was greater for the 500 μg of SEB/kg dose (44 h) than for the 1,500 μg of SEB/kg dose (14 h). Protection against lethality was conferred by 250 or 500 μl of anti-SEB/kg at the lower concentration of SEB, whereas the same antiserum dosage prolonged life but did not increase survival at the higher concentration of SEB. Questionable protection was afforded when 1,000 μl -

FIG. 6. Summary comparison of cumulative antibody reactive SEB antigen present throughout the course of experimental enterotoxemia in rats and monkeys. Monkeys were injected intravenously with either 1,500 or 500 μg of SEB/kg while rats received 1,000 $\mu\text{g}/100$ g. Specific anti-SEB antiserum was administered 1 h later when measured circulating toxin concentration was less than 3% of the injected dose (1, 9). The differences in serum hemagglutination titers between saline-injected and toxin-injected animals were used to estimate the amount of antibody reactive toxin antigen.

TABLE 2. Summary of monkey survival following challenge with SEB when different amounts of anti-SEB antibody were administered 1 h after toxin challenge

SEB ($\mu\text{g/kg}$)	Anti-SEB ($\mu\text{liters/kg}$)	Survival/total	Mean time to death (h)	Log ₂ titer ^a
500	0	0/2	44	0
500	250	1/1		(0)
500	500	2/2		(5) (7)
1,500	0	0/2	14	0, 0
1,500	100	0/1	64	0
1,500	250	0/2	56	0, 0
1,500	500	0/2	60	8, 8
1,500	1,000	1/4	73 ^b	7, (9)

^aTiters in parentheses are 48-h titers in surviving animals; titers not in parentheses are titers obtained at death or at 48 h in nonsurviving animals.

^bAverage of two animals excluding one animal who died at 8 days.

ers/kg was given ($\frac{1}{4}$ survived but one animal lived 8 days before death). There were no apparent differences in 48-h titer in those animals which survived and those which died. However, no survival was observed when the 48-h titer was zero except for the one animal challenged with 500 μg of SEB/kg and given 250 μliters of anti-SEB/kg. In this animal, the titer reached zero at 12 h; thus as much as 84% of the SEB regaining access to the extracellular space may have been neutralized by the antiserum.

DISCUSSION

Hemagglutination has been used to quantitate circulating antibody (5), and hemagglutination inhibition has been used to detect enterotoxin present in staphylococcal cultures (7). In the present experiments, the decrease in hemagglutination titer observed in vivo in the presence of toxin antigens might arise either by removal of antibody as an immune complex or by inhibition of antibody reactivity by antigen in analogy to hemagglutination inhibitor. By administering a given amount of antiserum to animals lacking hemagglutinating anti-enterotoxin antibodies, a consistent circulating titer was achieved in normal animals which varied only slightly over a 24-h period. This stability of titer in normal animals together with a consistent drop in titer when animals were injected with graded quantities of enterotoxin suggested that changes in antibody titer during toxemia could be used to measure the small quantities of enterotoxin antigen that might reappear in the extracellular space after initial tissue deposition. Accordingly, the type-

specific antiserum was administered 1 h after toxin injection because enterotoxin is rapidly removed from the circulation and by this time the toxin concentration in serum should be less than 3% of the injected dose (1, 9). By prolonging survival, this antiserum permitted measurement of the exchange of toxin antigens between tissues and the extracellular space over an extended period of time when the animals normally would have succumbed to shock.

The assumption that a progressive fall in titer indicated increasing availability of antigen was tested by comparing the hemagglutination pattern produced in animals receiving a single SEB injection to that produced by injecting the same total SEB concentration in divided doses. However, reduction of hemagglutination titer by specific antigen implies only that the antigen (whole toxin or fragments) is available to react with antibody. Since antibodies do not readily penetrate intact mammalian cells (3), antigen present within cells should not combine with antibody. Accordingly, reactive SEB antigen must be present either on cell surfaces, in the blood vascular compartment, or in the interstitial spaces. The slight fall in titer observed in control animals over a 24-h period may be due to equilibration of antibody between the intravascular and interstitial compartments. To correct for this change, control animals not receiving enterotoxin were compared to enterotoxin-injected animals, and the difference in response was used as an index of cumulative antigen available to antibody. Since antibody exchange between intravascular and interstitial compartments progressed slowly, a pronounced drop in serum titer would most likely be due to toxin antigens returning to the circulation rather than to the interstitial space. The validity of this interpretation rests on the assumption that during enterotoxemia there is not a change in vascular permeability to antibody. This assumption is consistent with the observations that measurement of body fluid compartments in unanesthetized rhesus monkeys given SEB have failed to demonstrate major shifts of volume during toxemia (12), that enterotoxin shock is due to a pooling of blood in peripheral vessels (2), and that the administration of antibody itself tends to abort the toxic effects of SEB.

Two hours after toxin challenge, the present methods revealed antibody-reactive toxin equivalent to 5 $\mu\text{g}/100\text{ g}$ for rats at a challenge dose of 1,000 $\mu\text{g}/100\text{ g}$ and 15 $\mu\text{g/kg}$ for monkeys at a challenge dose of 500 $\mu\text{g/kg}$. The corresponding values for the direct measurement of SEB in the circulation are 11 $\mu\text{g}/100\text{ g}$ of rat

body weight by I-125 tagging (9) and 7.0 $\mu\text{g}/\text{kg}$ of monkey by radioimmunoassay (1). The difference between experiments may be accounted for by the fact that free iodine is returned to the circulation after injection of I-125-tagged toxin which might result in detected label greater in amount than actual toxin concentration; on the other hand, the present method based on serum hemagglutination titers may measure some toxin present on surface membranes not available for direct blood quantification but nonetheless reactive to antibody.

Intravenously injected toxin is rapidly cleared from the circulation of both rats and monkeys principally by glomerular filtration (9). The filtered toxin is then reabsorbed by proximal tubular cells so that 1 h after toxin injection the majority of toxin is present within kidney cells. The liver and, to a lesser extent, the lung are alternate sites of toxin deposition (9). Studies of rat kidneys at different times after toxin injection indicated that the majority of the toxin present in the tubular cells was catabolized within 8 h (10). Similar information is not available for monkeys, but the data presented indicate that some of the toxin present in monkey tissues and presumably the kidney regains access to the circulation. This effect is greater in monkeys than in rats and implies that, if kidneys catabolize the toxin, this process may be less efficient in monkeys than in rats.

Because specific antibody appropriately administered after enterotoxin prolongs survival and may prevent lethality, the toxin which ultimately regains access to the extracellular space may be important to the pathogenesis of generalized enterotoxemia. Alternatively, the important pathogenetic toxin may be that toxin which resists rapid clearance from the circulation or is held on the surface membranes available to antibody. If so, the relative resistance of the rat to enterotoxin may be due either to more complete toxin removal from the circulation, more complete endocytosis of surface-held toxin, or to the fact that virtually no toxin once removed regains access to the extracellular space. In monkeys there appears to be a hyperbolic relationship between accumulating toxin antigen and time (Fig. 6) so that most toxin which regains access to antibody does so in the first 24 h. Such toxin may remain attached to cell surfaces or be free in the circulation where it might again be rapidly cleared, possibly to new tissue sites.

If the toxin dose is high enough, animals may die despite administration of protective antibody within the first hour of challenge. Such animals may die with demonstrable hemag-

glutinating antibody in the circulation at the time of death. However, the antiserum does affect the course of the toxemia because animals receiving antibody survive longer than those not receiving treatment. If lower concentrations of SEB are administered, the antiserum prevents lethality. Collectively, these observations suggest (i) that high concentrations of toxin in tissues may be slowly lethal and (ii) that SEB antigen which regains access to circulating antibody together with antigen remaining in the circulation or on cell surfaces 1 h after toxin challenge is essential to low-dose toxicity and to the accelerated course of toxemia in high-dose intoxication. If a redistribution of toxin from the kidney to other vital areas occurs, such a thesis would be consistent with the effect of antibody as well as the apparent lack of a determinant role for the kidney in the pathogenesis of enterotoxemia (6, 14). Since the majority of intravenously injected toxin localizes in renal tubular cells, it is probable that the demonstrated catabolism of toxin in tubular cells (10) serves to protect the host, although in a susceptible animal such as a monkey some toxin antigens may be returned to the circulation. As yet, the site(s) of lethal toxin action are still undetermined.

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